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See Attachment

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Report Title

Multiscale Problems in Circadian Systems Biology: From Gene to Cell to Performance

ABSTRACT

See Attachment

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
2012/03/22 1	13	Stephanie R. Taylor, Rudiyanto Gunawan, Linda R. Petzold, Francis J. Doyle III. Sensitivity Measures for Oscillating Systems: Application to Mammalian Circadian Gene Network, IEEE Transactions on Automatic Control, (01 2008): 0. doi: 10.1109/TAC.2007.911364
2012/03/22 1	12	Yongqiang Wang, Francis J. Doyle. On influences of global and local cues on the rate of synchronization of oscillator networks, Automatica, (06 2011): 0. doi: 10.1016/j.automatica.2011.01.074
2012/03/22 1	11	S.R. Taylor, H.P. Mirsky, R.A. Harvey, J. Stelling, F.J. Doyle. Distribution-based sensitivity metric for highly variable biochemical systems, IET Systems Biology, (01 2011): 0. doi: 10.1049/iet-syb.2009.0064
2009/09/15 1	9	Wenxue Wang, Christian Cajochen, Scott T. Grafton, Francis J. Doyle III. Modeling Circadian -Dependent Learning Performance of Sequence Structures, FOSBE Conference, (2009): . doi:
2009/09/15 1	8	Neda Bagheri, Stephanie R. Taylor, Kirsten Meeker, Linda R. Petzold, Francis J. Doyle III. Synchrony and Entrainment Porperties of Robust Circadian Oscillators, Interface Focus, (2008): . doi:
2009/09/15 1	7	Richard Harang, Kirsten Meeker, Guillaume Bonnet, Francis J. Doyle III, Linda Petzold. Period Distribution In Mammalian Circadian Neurons, FOSBE Conference, (2009): . doi:
2009/09/15 1	6	Stephanie R. Taylor, francis J. Doyle III, Linda Petzold. Oscillator Model Reduction Preserving the Phase Response: Application to the Circadian Clock., Biophysical Society, (2008): . doi:
2009/09/15 1	5	Henry P. Mirsky, Andrew C. Liu, David K. Welsh, Steve A. Kay, and Francis J. Doyle III. A Model of the Cell-Autonomous Mammalian Circadian Clock., Proceedings of the National Academy of Sciences(US), (2009): . doi:
2008/10/29 0	2	Stephanie R. Taylor, Francis J. Doyle III, Linda R. Petzold. Oscillator Model Reduction Preserving the Phase Response: Application to the Circadian Clock, , ( ): . doi:
2008/10/29 0	1	Neda Bagheri, Stephanie R. Taylor, Kirsten Meeker, Linda R. Petzold, Francis J. Doyle III. Synchrony and Entrainment Properties of Robust Circadian Oscillators, , ( ): . doi:

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Received	Paper

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Number of Papers published in non peer-reviewed journals:

(c) Presentations

1. Foteinou P.T., J. Hogenesch and F.J. Doyle 3rd. Elucidating the Effects of SIRT1 on Circadian Amplitude: Insights from an in-silico Transcriptional-Enzymatic Model. CCB-UCSD Symposium, San Diego, CA, 2012.
2. Foteinou P.T. and F.J. Doyle 3rd. Modeling Circadian Interactions between Peripheral Clocks and Metabolism. AIChE National Meeting, Minneapolis, MN, 2011.
3. Ananthasubramaniam B., P.T. Foteinou, A. Sungwon, E.D.Herzog and Doyle F.J. 3rd, Modeling Interactions in Circadian Neurons and Peripheral Clocks. 12th Annual UC Systemwide Bioengineering Symposium, Santa Barbara, CA, 2011.
4. Taylor, S.R., F.J. Doyle 3rd and L. Petzold. Phase Response-Based Model Reduction Improves Analysis of Clock Models. Society for Research on Biological Rhythms, Sandestin, FL, 2008.
5. Meeker, K., L. Petzold, and F. Doyle 3rd. Synchronization in a Network of Circadian Oscillators. Society for Research on Biological Rhythms, Sandestin, FL, 2008.
6. Taylor, S.R., L.R. Petzold, and F.J. Doyle 3rd. Analyzing Circadian Networks with Parametric Impulse Phase Response Curves. Biomedical Engineering Society (BMES) Fall Meeting, Hollywood, CA, 2007.

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Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

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TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received		Paper
2012/03/22 1(	14	Felipe N□unez, Yongqiang Wang, Andrew R. Teel, Francis J. Doyle III. Bio-inspired synchronization of non-identical pulse-coupled oscillators subject to a global cue and local interactions, 4th IFAC Conference on Analysis and Design of Hybrid Systems, 2012. 2012/06/08 03:00:00, . : ,
2012/03/22 1(	15	Yongqiang Wang, Francis J. Doyle III. The exponential synchronization of Kuramoto oscillator networks in the presence of combined global and local cues, American Control Conference, 2011. 2011/06/29 03:00:00, . : ,
2012/03/22 1(	17	Yongqiang Wang, Francis J. Doyle. The synchronization rate of oscillator networks subject to delayed and directed interaction, 2010 48th Annual Allerton Conference on Communication, Control, and Computing (Allerton). 2010/09/28 03:00:00, Monticello, IL, USA. : ,
2012/03/22 1(	16	Yongqiang Wang, Francis J. Doyle. The influences of global and local cues on the synchronization rate of interconnected oscillator networks subject to time delays, 2010 49th IEEE Conference on Decision and Control (CDC). 2010/12/14 03:00:00, Atlanta, GA, USA. : ,

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(d) Manuscripts

Received		Paper
2008/10/29 0(	3	Stephanie R. Taylor, Neda Bagheri, Kirsten Meeker, Linda R. Petzold, Francis J. Doyle III. Robust Timekeeping in Circadian Networks: From Genes to Cells, ( )

TOTAL: 1

Number of Manuscripts:

Books

Received Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Richard S.H. Mah Lectureship, Northwestern University, 2012

David Himmelblau Award (AIChE CAST Division), 2011

IFAC Best Survey Paper Award, J. Process Control, 2008-2011

Fellow, AAAS, 2009

Zaborszky Distinguished Lectureship, Washington U., 2010

ASEE Lectureship Award, Chemical Engineering Division, 2010

Harry Nicholson Distinguished Lectureship in Control, Sheffield University, 2010 (Inaugural Lecturer)

Distinguished Member Award, IEEE CSS, 2009

Fellow, AIMBE, 2009

Fellow, IFAC, 2009

Distinguished Lecture, Padova University, Italy, 2009

Fellow, IEEE, 2008

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Peter St. John	0.02	
FTE Equivalent:	**0.02**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Osman Shaik	0.30
YongQiang Wang	0.07
FTE Equivalent:	**0.37**
Total Number:	**2**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Bharath Ananthasubramaniam	0.38
FTE Equivalent:	**0.38**
Total Number:	**1**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment

Technology Transfer

Abstract

The circadian system or "biological clock" orchestrates the temporal organization of several physiological and metabolic processes over a 24-hour period in most living organisms. In mammals, the circadian timing system has a hierarchical organization composed of the central pacemaker in the suprachiasmatic nucleus (SCN) which coordinates the oscillating activity of peripheral clocks that are present in almost all tissues. At the molecular level, circadian oscillations in both SCN and peripheral cells are based on negative autoregulation of gene expression involving essentially the same core clock components. At the tissue level, the SCN consists of a coupled network of heterogeneous neurons that fire synchronously through neurotransmitter coupling and are entrained to 24-hour period through daily light-dark cycles. At the organismal level, the SCN synchronizes peripheral tissue clocks via hormonal and indirect cues such as feeding time, probably through modifications of metabolite availability. More recently, experiments have shown that interactions between circadian and metabolic systems are bidirectional in that metabolites can feedback into the clock. These findings emphasize the need of a multiscale integrative approach to understanding circadian regulatory processes. Here, we adopt a quantitative systems biology approach that uncovers critical properties of the circadian oscillator at various scales (neurons, SCN, periphery).

Keywords: computational and theoretical biology, biological rhythms, multiscale modeling, metabolism, synchrony and entrainment

Scientific Progress and Accomplishments

The circadian system or "biological clock" orchestrates the temporal organization of several physiological and metabolic processes over a 24-hour period in most living organisms from bacteria to plants and mammals. This highly conserved system enables the organism to adapt to common daily and seasonal changes, such as the day-night cycle and food availability. In mammals, the circadian timing system is composed of the central pacemaker, which resides within the suprachiasmatic nucleus (SCN) and coordinates the oscillating activity of peripheral clocks that are present in almost all tissues. At the molecular level, circadian oscillations in both SCN and peripheral cells are based on negative autoregulation of gene expression involving essentially the same core clock components. At the tissue level, the SCN consists of a coupled network of heterogeneous neurons that fire synchronously through neurotransmitter coupling and are entrained to 24-hour period through daily light-dark cycles. At the organismal level, the SCN synchronizes peripheral tissue clocks via humoral signals and indirect cues such as feeding time, probably through modifications of nutrient availability. More recently, experiments have shown that interactions between circadian and metabolic systems are bidirectional in that the clock not only influences metabolic processes but also metabolic signals feedback into the circadian machinery. Taken together, the circadian system has a hierarchical, multiscale organization and integrative quantitative approaches are crucial to understanding circadian regulatory processes. Here, the opportunities of a multiscale integrative approach will be discussed for systems-level analysis and modeling in circadian physiology. The unique aspects of this work, which are discussed in great detail in the relevant publications [1-9], are summarized below and include:

(i) development of a cell autonomous (single cell) mathematical model of a circadian neuron which resolves the experimentally observed discrepancies between the tissue scale and the cellular scale;
(ii) generation of a tissue level model by coupling circadian neurons using the vasoactive intestinal polypeptide (VIP) predicting tissue-level phenotypes;
(iii) development of nonlinear model predictive control strategies to control circadian behavior;
(iv) development of novel sensitivity and model reduction techniques used to assess both the robustness of the model to parameter perturbation and reduce the dimensionality scale for computational feasibility;
(v) development of “integrate-and-fire” models to represent synchronized population-scale phenomena in many other biological systems including the annual spawning of certain species of corals and;
(vi) derivation of rigorous analytical metrics to understand general principles of biological synchrony and circadian entrainment.

Our most recent activities continue to probe at the sources of regulation that give rise to robust oscillations and highly precise periods in the mammalian “biological” clock.

Firstly, upon recognition of the reciprocal interaction between the circadian clock and metabolism, we have developed a novel circadian-enzymatic model that quantifies the metabolic regulation of the core circadian oscillator. Such model generates new experimentally testable hypotheses that can explain a contradiction in the metabolic/clock connection. *Secondly*, we have also begun to obtain theoretical results in the regulation of the collective period in a coupled circadian oscillatory network. Using an autorepression-based gene regulatory network, such analytical insights indicate that in the presence of a coupling delay, the strength of the coupling (intercellular) neurotransmitter regulates the collective period. Further details of our previous and ongoing research activities are discussed below.

Modeling cell-autonomous oscillations of a circadian neuron

The fundamental structure of the biochemical circadian oscillator in mice has been known for roughly 2-3 decades. This has led to the construction of various mathematical models of the mammalian clock, both deterministic and stochastic. These models were constructed from tissue-level data and therefore describe the behavior of the molecular clock in communicating cells. Recent experiments, however, show that the gross phenotype of the system (i.e., rhythmicity vs. arrhythmicity) is often different in gene knockouts between autonomous and non-autonomous cells [10].

To maintain proper functionality, individual clock components must rise and fall in fixed phase relationships with one another, alternately turning on and off the genes in the network. We built a network structure [5], **Figure 1**, from the most recent experimental data, incorporating eight key genes – more than are found in any previous mathematical model of the mouse clock. We then used the known phase relationships among mRNAs and between mRNAs and proteins, **Figure 2**, to construct a cost function and found a best-fit parameter set for the model by minimizing this cost through an iterative evolutionary strategy. The resulting model was validated against the gross phenotypes of seven gene knockouts and two double gene knockouts. For all nine cases, the model correctly predicts the phenotype. Additional validation was achieved via comparison to the known molecular biology of the system.

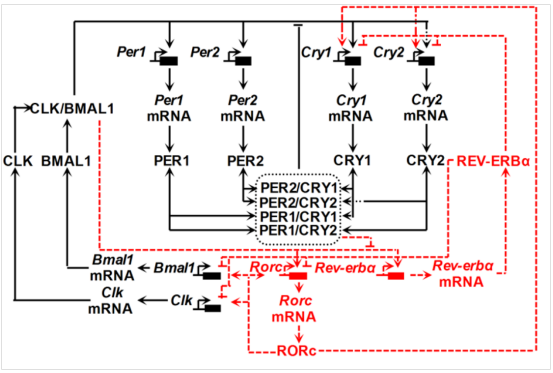

Figure 1: Network topology of a cell-autonomous circadian neuron. Interacting components involve core clock feedback loops (e.g. PER/CRY) and secondary (auxiliary) loops (e.g. ROR/REV-RB) regulating the circadian clock.

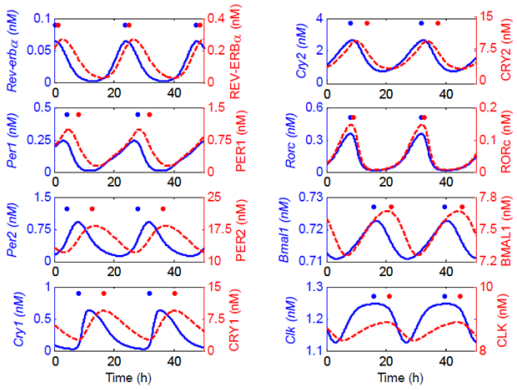

Figure 2: Dynamic profiles of the mammalian circadian clock. Blue and red dots represent experimentally observed phase relationship between mRNA and proteins respectively.

Further, we assessed the robustness of our wild-type model to maintain periodicity in response to parameter perturbation, a requirement to ensure that our parameterization is not so restrictive as to be unrealistic. We found that the model behavior is maintained in 98% of alternative models. Taken these together, although this model was calibrated using only the phase relationships among mRNA and protein concentrations, it accurately predicts all the single cell phenotypes of gene knockout mutations for which experimental data exist.

Tissue-level modeling of coupled autonomous circadian neurons

While the gene-regulatory feedback networks in each SCN pacemaker produce "noisy" self-sustained rhythms, robust approximately 24-hour output is generated from the SCN through the coupling of thousands of pacemakers through neurotransmitter coupling and is entrained to 24 hour period through daily light-dark cycles.

Synchronization among neurons in the SCN is achieved, at least in part, through circadian release of the VIP and its reception by the VPAC2 receptor, leading to the initiation of a signal cascade involving activation of G-protein, internalization of the VPAC2 receptor, activation of adenylate cyclase, production of cyclic AMP (cAMP), activation of protein kinase A (PKA), phosphorylation of cAMP-response element binding protein (CREB), and finally, up-regulation of one or more Per genes. Circadian oscillations have been observed in cAMP and calcium levels and also in CREB-mediated gene expression in the SCN. Light, the principal agent responsible for entrainment of the internal clock to the environment, is transduced on the retina and channeled to the SCN via the retinohypothalamic tract, where its effects ultimately lead to up-regulation of one or more period (Per) genes. The path that leads to light-induced up-regulation may be similar or identical to that which synchronizes the SCN neurons to one another, since VIP release appears to be enhanced by light.

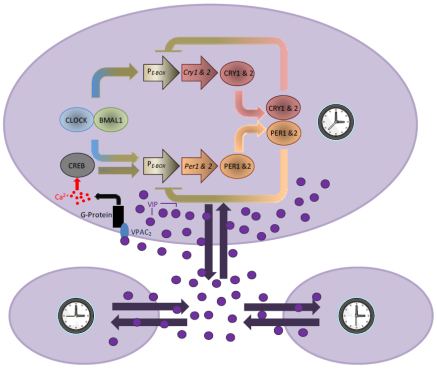

Figure 3: Schematic of the coupled (tissue-level) model of the mammalian circadian clock. Communication among cells is achieved via circadian release of the vasoactive intestinal polypeptide (VIP), which is received and transduced by the VPAC2 receptor. Figure is adapted from [11].

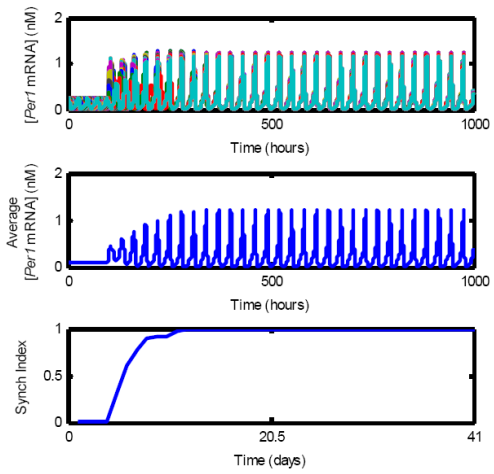

Figure 4: Time courses for a 5 x 5 grid of coupled cell-autonomous circadian clocks. Top plots show Per1 mRNA concentrations for all 25 cells, middle plots show average Per1 mRNA concentration across all 25 cells, and bottom plots show synchronization indices (SIs).

We incorporated the signaling cascade described above and shown in **Figure 3** using the mechanistic representation used by To *et al.* [12] to couple a 5x5 grid of our cell autonomous model in Mirsky *et al.* [5]. We found parameters for the 22 parameters in the VIP cascade that produced circadian oscillations in the tissue-level model while retaining all the original parameters for the cell autonomous model. These oscillations (see **Figure 4**) are produced by synchronized oscillations in the 25 cells with a period of 30 hours and moreover, these oscillations are robust in the presence of parameter heterogeneity in the coupled cells. As mentioned earlier, the cell autonomous model can predict all the single-cell knockout phenotypes observed in experiments. However, some knockout phenotypes at the tissue-level are different from the single-cell phenotypes [10]. Unfortunately, the deterministic model showed lack of oscillations at the current parameter values with Per1, Per2 and Cry1, which give robust rhythms in the tissue. Nevertheless, we tested the knockdown (instead of knockout) of these 3 genes and were still able to obtain rhythmic behavior as seen experimentally.

Synchrony and entrainment properties of robust biochemical oscillators

In addition to mathematical modeling (see above), we also employed advanced systems theoretic tools (e.g. control systems analysis algorithms) in order to better understand, characterize and control the circadian timekeeping system [2, 3]. Precise synchronization of these rhythms is an essential part of circadian organization, which is hierarchically organized into the following steps: (i) periodic light detected by the eyes entrains (synchronizes) ~20,000 neurons in the SCN via neuronal signals; (ii) within SCN tissue, individual neurons synchronize each other via VIP coupling and/or gap junctions and subsequently (iii) the SCN synchronizes the other peripheral tissue clocks via humoral/indirect signals and

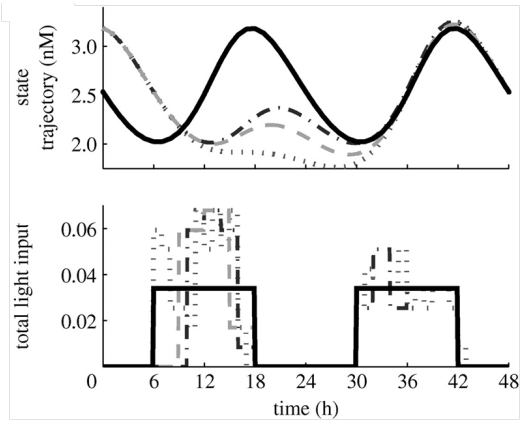

Figure 5: State dynamics and light profiles used for circadian entrainment. (Top) State dynamics as they converge to the reference trajectory (bold solid line); (Bottom) Light profiles of duration 1 (dotted lines), 2 (dot-dashed lines) and 3 hours (dashed lines) used to reset the phase differences; these sequences also converge to nominal light/dark cycles (bold solid line).

such interaction is reciprocal in nature. Since little is known about this communication, our study investigated the coupling mechanisms that give rise to synchronized networks in the SCN. Using deterministic and discrete stochastic models in both the single-cell and network setting, we investigated optimal control strategies for light-induced circadian phase entrainment as illustrated in **Figure 5**. Specifically, we tested the efficacy of the optimization (model predictive control, MPC) algorithm with respect to various control (light) inputs as applied to a nonlinear mammalian model. Further, our investigation of the *Drosophila* clock model revealed that only 82 (out of 960) cellular coupling mechanisms would provide synchrony and therefore significantly decreasing the number of candidates to be tested experimentally. Thus, the analysis of phase characteristics may facilitate the identification of relevant coupling mechanisms for the ensemble of noisy (stochastic) circadian clocks.

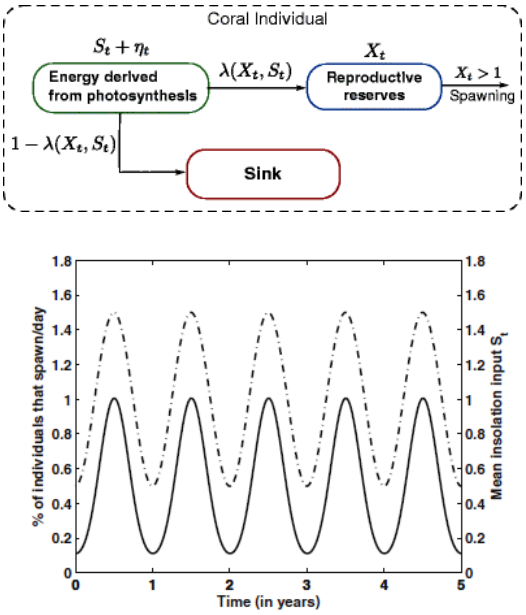

Figure 6: A bioenergetic integrate-and-fire model of coral spawning. (Top) Allocation to the reproductive reserves X_t of energy obtained by a coral individual from its photoautotropic symbiont derived from insolation S_t with fluctuations n_t with the remaining energy going toward growth, somatic maintainance and other reserves. $\lambda(X_t, S_t)$ represents the fractional allocation toward reproduction; (Bottom) The percentage of the individuals in a colony that spawn in a day for a 5-year period for the simple reserve accumulation under $\lambda(X_t, S_t) = S_t$.

Recently, our research efforts in systems biology have also expanded into the application domains of ecology, with the broad technical theme of understanding synchronized population-scale phenomena, such as coral spawning, using coupled and driven oscillator models [1, 4]. These studies identify the sources of coherence in the annual spawning of certain species of coral, and this builds on the group's expertise in robustness analysis of coupled oscillators in the brain that are responsible for circadian rhythms. More specifically, we developed a bioenergetic model integrate-and-fire model, **Figure 6** that reveals how annual insolation rhythms can entrain the gametogenetic cycles in tropical hermatypic corals to the appropriate spawning season since photosynthate is their primary source of energy. In the presence of short-term fluctuations in the energy input, a feedback regulatory mechanism is likely required to achieve coherence of spawning times to within one lunar cycle, in order for subsequent signals such as lunar and diurnal light cycles to determine the "correct" night of spawning. The feedback mechanism can also provide robustness against population heterogeneity that may arise due to genetic and environmental effects.

Novel sensitivity techniques for highly variable biochemical systems

Classical sensitivity and model reduction techniques are long-standing systems biology approaches used both to gain insight into model subprocesses and reduce the computational cost of simulation and analysis. For systems with periodic and stochastic behavior the situation is somewhat challenging. Recently, we developed a distribution-based sensitivity analysis method [8] that addresses the robustness of a model (system) to extrinsic noise in parameters in both deterministic and stochastic regimes. We particularly applied this method to a highly nonlinear system, the *Drosophila* biological clock. As seen in **Figure 7** the fragility of this cell autonomous model is concentrated in processes whose rates are expressed in parameters that are not clock specific (e.g., general transcription, translation), while the clock is robust to perturbations in rates of reactions that are found only in the clock network (e.g., association and dissociation of clock proteins that form heterodimers). Remarkably, in spite of being a highly nonlinear system, the circadian clock produced proportional larger deviations for increasing parameter perturbations. Finally we showed that the circadian clock is quite insensitive to extrinsic noise in the form of fluctuations in the number of cellular components that alter reaction rates at multiple genes and gene products.

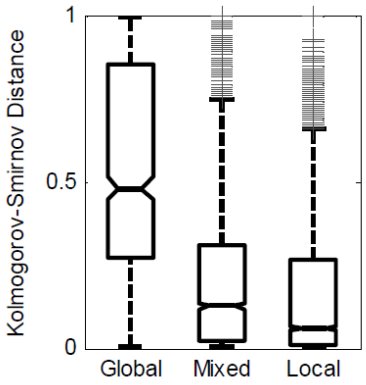

Figure 7: Box plots of sensitivities classed by parameter type. Global parameters are those that are not clock-specific (e.g., general transcription, translation), local parameters are clock specific (e.g., association and dissociation of clock components), and mixed parameters have characteristics of the other two classes. Note that the clock is principally sensitive to perturbations in global parameters.

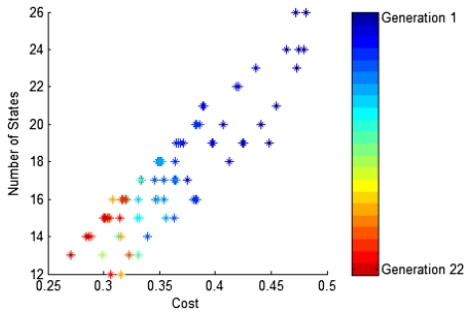

Figure 8: Oscillator model reduction preserving the phase response behavior. Cost and model size of the 10 fittest individuals from each 22 generations created using a genetic algorithm hybridized with a local deterministic search. The earliest generations are shown in blues and the latest are shown in reds.

In addition to sensitivity analysis, we also developed a novel model reduction technique for circadian models that preserves the phase response behavior, a key performance criterion for biological clocks [6, 9]. In our previous studies [2, 3] we have already shown that phase entrainment can serve as a critical control objective to investigate dynamics both at the single cell level and at the population level using coupled oscillators. The proposed methodology effectively reduced Forger and Peskin's 61-state mammalian model [13] to 13 states – a fivefold reduction in size as illustrated in **Figure 8**. Specifically, we discovered that four feedback loops are unnecessary (redundant) with respect to the phase response behavior of the single cell oscillator. Using a coupled multioscillator circadian

model, we further demonstrated that by preserving the phase response properties of a single oscillator, the timing behavior at the population level is also preserved.

Modeling the reciprocal interaction between circadian timekeeping and metabolism

In the last decade, many aspects of the molecular mechanism of circadian rhythms have been elucidated, thanks to the advanced progress in molecular biology experiments. While it has been a dogma for years that the circadian clock is regulated by transcription feedback loops, emerging studies now highlight the importance of another loop, an enzymatic one. Of particular interest are recent groundbreaking studies [14, 15] showing that the NAD^+-dependent enzyme SIRT1 functions as a histone deacetylase that regulates core clock components including the proteins BMAL1 and PER2. While these studies show that the histone deacetylase SIRT1 is involved in regulating the circadian amplitude, they differ in the response of gene expression to loss of SIRT1 as recently reviewed in [16]. Predicated upon this apparent contradiction, which we refer to as "*SIRT1 paradox*", this study aims to explore such paradoxical effects through the development of a novel circadian-enzymatic model, schematized in **Figure 9(left)**, with the perspective of generating new experimentally testable hypotheses.

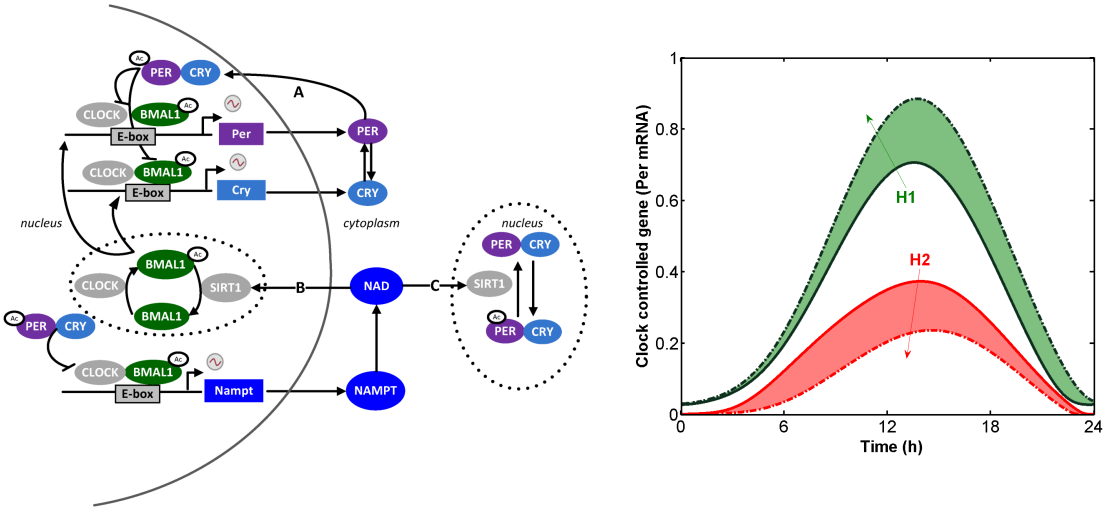

Figure 9: (Left) Network topology of the circadian-enzymatic model. Upper case letters represent the interlocked transcriptional and enzymatic feedback loops exerted by the canonical PER-CRY loop (A) and the enzymatic NAD loop which regulates the deacetylation of BMAL1 activator (B) and PER-CRY repressor (C); **(Right) Model recapitulates the experimentally observed differential amplitude response due to lack of SIRT1**. Simulations were generated using two independent parameters sets. Solid lines represent the wild type condition while dashed lines represent SIRT1 null mutation. Parameter set H1 was selected because the circadian amplitude increases in response to SIRT1 deficiency, while parameter set H2 corresponds to a situation in which the amplitude decreases in the $SIRT1^{-/-}$ mutant.

The performance of the resulting model is evaluated through its potential to capture experimentally observed circadian phenotypes of gene knockouts including circadian amplitude variation due to loss of metabolic activity (e.g. $SIRT1^{-/-}$), **Figure 9(right)**. Interestingly, the model recapitulates the differential amplitude response due to lack of SIRT1 as experimentally shown in [14, 15]. Specifically, the high-amplitude circadian oscillations as shown by Nakahata *et al.* [15] is simulated for stoichiometric ratios between the activator and the repressor that are comparatively smaller than those giving

rise to the reduced circadian amplitude as reported by Asher *et al.* [14]. In this case (*H2* phenotype) such high ratios are attributed to higher levels of the acetylated activator and lower levels of the acetylated repressor complex. This demonstrates the crucial role a genetic variation in the background levels of the acetyltransferases – enzymes regulating the acetylation levels of BMAL1 and PER proteins – might play in modulating their stoichiometry and thereby the amplitude phenotype under null enzymatic mutations (e.g. $SIRT1^{-/-}$). Interestingly, upon saturating the effect of acetylation on PER while reducing the acetylation of BMAL1 the model predicts increased rather than reduced amplitude in the absence of SIRT1. Under these conditions the effect of SIRT1 deficiency on the negative (PER) component becomes negligible and its effect on the positive (BMAL1) limb dominates.

Experimentally, a decrease in BMAL1 acetylation would be analogous to a decrease in CLOCK gene expression which elicits BMAL1 acetylation. In regard to the saturation effect of PER acetylation, a possible scenario would be to overexpress the enzyme that acetylates PER protein. Given that such an enzyme is not known the model suggests an alternative scenario that involves a reduction in the degradation rate of PER protein. Since the kinase (CKI) promotes proteasomal PER degradation, reducing CKI levels would be equivalent to reducing the degradation of PER protein. As part of this study and in collaboration with colleagues at UPenn, we aim to conduct the experimental verification experiments using the RNA interference (RNAi) technology.

Intercellular coupling strength regulates the collective period of delay-coupled biological oscillators

Most biological rhythms are generated by multiple inter-coupled cellular oscillators that operate in synchrony. Experimental studies showed that in the presence of a coupling delay, the collective period can differ significantly from the individual oscillation period. The phenomenon is intriguing in that the period of a biological clock is of vital importance and is fundamental to many biological functions. Autorepression-based gene regulatory oscillator is thought to be the core of many biological clocks such as cell cycles and the vertebrate segmentation clock, and it is also thought be to at the heart of multiple circadian systems. In such gene regulatory oscillators, the protein directly binds to the regulatory DNA of its own gene to inhibit transcription, with an explicit delay involved in the synthesis of mRNA and protein (c.f. **Figure 10**). Based on delay-coupled autorepression oscillators, we studied analytically the influence of intercellular coupling strength on the collective period. Consistent with previously published models, the intercellular coupling is assumed to be of a diffusive type.

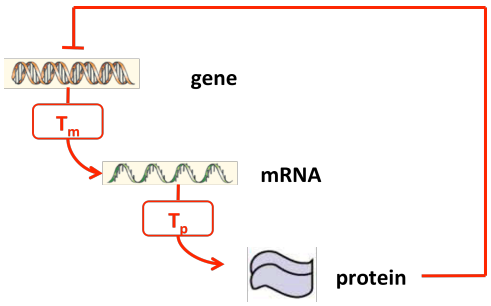

Figure 10: The schematic of autoregression based gene-regulatory oscillators. T_m and T_p denote the time delay in transcription and translation, respectively.

We prove analytically that in the presence of a coupling delay, the strength of intercellular coupling regulates the collective period. More specifically, we prove that depending on delay magnitude, the collective period can increase or decrease with the coupling strength. We also give the explicit specific region in parameter space in which the collective period increases/decreases with the coupling strength. To our knowledge, this is the first time that the collective period of coupled mechanism-based oscillators is analytically obtained and is rigorously proven to be regulated by the intercellular coupling strength. Moreover, the collective period is analytically obtained in a closed-form, which has not been reported in the literature. The results are given in terms of biochemical parameters, and may give guidance in synthetic biological oscillator design. The prevalence of autorepression in biological oscillations supports the potentially broad application to other biological clocks.

We utilize the experimental data [17] of the segmentation clock in zebrafish embryos to verify our theoretical predictions. The segmentation clock controls the somitogenesis in vertebrate development. It is composed of many cellular gene regulatory oscillators [18]. These cellular oscillators are located within the posterior mesoderm (PSM) and Delta-Notch signaling synchronizes the cellular network (c.f. **Figure 11**). Treating wild-type zebrafish embryos with DAPT, a γ-secretase inhibitor that suppresses Notch receptor function, will attenuate coupling strength but will not influence the time delay in Delta-Notch coupling [17]. Using our theoretical results, we can analytically predict the collective period under the treatment of different DAPT concentrations, as shown by the red dashed line in **Figure 11(left)**. The experimental measurement of collective period under different DAPT concentrations is borrowed from [17] and is represented by green squares and error bars in the **Figure 11(right)**. The analytical prediction is in very good quantitative agreement with the experimental data.

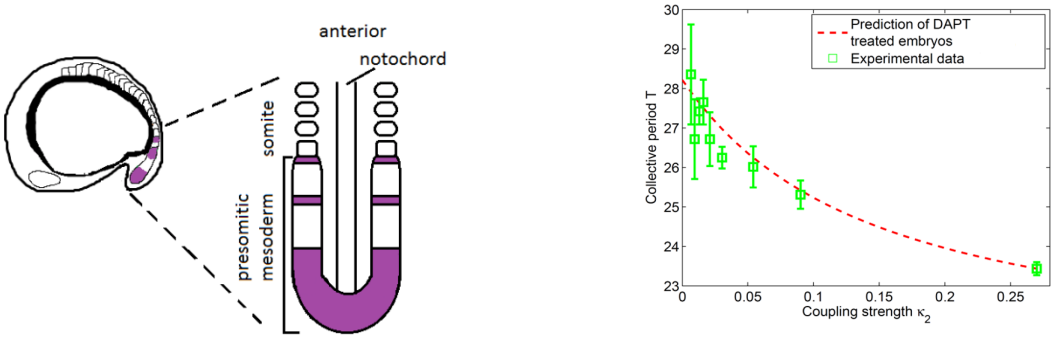

Figure 11: (Left) Schematic of the presomitic mesoderm (PSM). The purple area indicates waves caused by the periodic expression of the *her* gene; **(Right) Experimental confirmation of the theoretical results.** The coupling strengths (κ_2) under different concentrations of DAPT treatment are calculated based on the linear relation between coupling strength and Notch signaling level and the fact that DAPT suppresses Notch signaling following Michaelis-Menten kinetics [17]. The experimental data of wide-type zebrafish embryos treated with different DAPT concentrations are represented by green squares and error bars. The analytic prediction is in very good quantitative agreement with the experimental data.

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